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Laurie Scheiner
Art Unit 1648
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Pending Nucleic Acid and/or Pending Amino Acid database searches now generate two set
results. These databases were split into to two parts to reduce the time needed to update the
databases daily. The split freed up more machine time for processing searches.

Searches run against the Nucleic Acid Pending database produce two sets of results, with th
extensions, **.rnpm** and **.rnpn**

Searches run against the Amino Acid Pending database produce two sets of results, with the
extensions, **.rapm** and **.rapn**

The Pending database search results should not be left in the case because they contain data that is confidential.

According to the Pre Publication Rules, every patent application received by the United States Patent and Trademark Office after November 29, 2000 will be pre-published at eighteen months from the effective filing date. When the application is published the contents, including the sequences, will become prior art.

Two new databases have been created to hold the pre-published sequences:
Published_Applications_NA contains nucleic acid sequences; the search results will have the extension **.rnpb**.
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Each pre-published application is given a unique Publication Number. An example of a Publication Number is US20021234567A1. The "US" indicates the application was a U.S. application. The first 4 digits show the calendar year the application was published. The next 7 digits represent when the application was published. This 7-digit number starts at zero at the beginning of each calendar year. Each application published is given the next number in order. The "A" indicates a utility patent application and the "1" shows that this was the first time the application had been published. If the applicants submit changes to the application, they may requests that the changed application be published again. In such instances, the "1" at the end of the number would be replaced by a "2".

Sequences in the PGPub database are public information; it is permissible to leave these results in the case.

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Ebringer, A. et al., Environmental Health Perspectives, Vol. 105, No. 11, November 1997 (1997-11), pages 1172-1174.
Thanks!

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Bovine Spongiform Encephalopathy: Is It an Autoimmun Diseas Due to Bacteria Showing Molecular Mimicry with Brain Antigens?

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Bovine spongiform encephalopathy (BSE) could be an autoimmune disease produced following exposure of cattle to feedstuffs containing bacteria showing molecular mimicry between bacterial components and bovine tissue. Analysis of molecular sequence databases (Genbank and SwissProt) shows that three bacteria (*Acinetobacter calcoaceticus, Ruminococcus albus,* and *Agrobacter tumefaciens*) share sequences with the encephalitogenic peptide of bovine myelin, while three molecules in *Escherichia coli* show molecular mimicry with host-encoded prion protein. Immune responses against these bacteria at both T and B cell levels may cause neurological tissue injury resembling BSE. The role of these bacteria in BSE, if any, merits further investigation. *Key words:* autoimmune disease, bovine spongiform encephalopathy, Gram-negative bacteria, molecular mimicry.

Environ Health Perspect 105:1172–1174 (1997). *http//ehis.niehs.nih.gov*

The relative increase in the late 1980s of bovine spongiform encephalopathy (BSE) in cattle in the United Kingdom has evoked some public interest. It appeared that this increase occurred after feeding cattle with ovine/bovine material, although since the practice has been discontinued, the number of BSE cases has steadily declined (*1*).

Several theories have been proposed to explain this phenomenon, the most prominent being the prion hypothesis (*2*).

The prion hypothesis postulates that there is an infectious particle of a virus/prion nature which is transmitted to sheep (scrapie) and cows (BSE) and maybe even humans (Creutzfeldt-Jakob disease)(CJD). However, there are several difficulties with this hypothesis.

- There is no structural evidence for the presence of the particle: there are no electron microscopy pictures of such an agent, there is no immunological evidence of infection, and there are no microbiological methods available to grow such a virus/prion agent (*3*).
- The prion sequence is actually encoded by the host (*4*) and is therefore a self-protein and probably not part of an external infectious agent.
- The human prion sequence that accumulates in brain lesions, KTNMKHMAGAAAAGAVVGGLG, consists mostly of aliphatic amino acids, which readily polymerize into amyloid like fibrils (*5*). This could explain why these self-proteins are relatively resistant to hydrolysis by macrophage enzymes and therefore would accumulate in neurological lesions following nerve damage.
- The proposal that the prion agent consists only of self-replicating proteins (the protein only hypothesis) (*6*) and is devoid of nucleic acids (*7*) raises serious problems in molecular biology (*8*).
- Furthermore immunodeficient animals, such as SCID mice, do not develop scrapie following scarification with affected brain tissue (*9*). It is most unusual to find absence of immune reactivity as protective since SCID mice readily succumb to viral and bacterial infections.

Experimental Allergic Encephalomyelitis as a Model of an Autoimmune Disease

The injection of xenogeneic antirabies vaccine into humans by Pasteur some 100 years ago led to many cases developing severe neurological disorders, which subsequently were considered to be due to contaminating brain antigens evoking immune responses in the host. In the 1920s, several animal models of experimental allergic encephalomyelitis (EAE) were described in which injections of brain tissue led to immune responses producing a variety of neurological disorders in the recipient animals (*10*). Injection of brain tissue leads to loss of muscle tone, an ataxic gait resulting in weakness of hind legs, which often progresses to total paralysis and finally death (*11*). Amounts as small as 0.1 µg of purified fractions of bovine brain tissue can cause neurological disorders in experimental animals such as guinea pigs (*12*). More recently, some children injected with human growth hormone, extracted from postmortem pituitary glands, developed a neurological disorder resembling EAE because of contamination with denatured brain tissue (*13*).

It is not outside the realm of the possible that bowel bacteria may carry antigens cross-reacting with brain tissue, which if present in high quantities could produce an immunological response in cattle resembling EAE. A possible mechanism of how this disease could be produced is illustrated by the human arthritic disorder ankylosing spondylitis (AS).

The onset of AS is usually between 15 and 30 years of age and is characterized by morning muscle stiffness, recurrent backache, and arthritis of large joints such as knees, hips, and ankles.

Ankylosing Spondylitis as an Autoimmune Disease Caused by Bowel Bacteria

Some 95% of patients suffering from AS possess the major histocompatibility complex (MHC) antigen HLA-B27, while the frequency of this MHC allele in the general U.K. population is about 8%. Studies on the bowel bacteria *Klebsiella* have shown that they possess molecules that cross-react with HLA-B27 (*14*). When these bacteria are present in high concentrations in the bowel flora, they evoke immune responses characterized by high titers of anti-*Klebsiella* antibodies, which also have an anti-HLA-B27 activity and therefore could be considered as autoantibodies (*15*). Elevated levels of antibodies to *Klebsiella* microbes have now been described in AS patients from 15 different countries (*16,17*). A possible explanation for high levels of these bacteria in the bowel flora is a high consumption of starch (*18*). A low starch diet has been used in the treatment of AS patients in an endeavour to reduce the quantity of *Klebsiella* microbes in the bowel flora (*19*).

BSE could have occurred by a similar mechanism, as the bone meal supplementary feeds were known to contain "green offal," which may have been inadvertently contaminated by bowel bacteria. Some of these bacteria may have contained antigens cross-reacting with brain tissue and thereby leading to the production of antibacterial antibodies with activity against various components of brain and prion tissues. The first cases of BSE were described in the early 1980s reaching a peak in 1988 when the use of supplementary feeds containing bonemeal was banned by the

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Ministry of Agriculture, Food and Fisheries (MAFF). Since the ban was introduced, the number of cases of BSE has drastically fallen and the possibility is emerging that the disease could be completely eradicated from U.K. herds (*1*).

The unresolved question remains as to why this ban has been successful: Was it due to the elimination of animals infected by prion particles? Or can another explanation account for these results?

The Hypothesis that BSE Is a Form of Autoimmune Disease

We propose the hypothesis that BSE is caused by cross-reactive autoantibodies evoked following exposure of cows to biological material from cows/sheep containing bacteria that may cross-react with bovine self-antigens. Since neurological damage is the main feature of BSE, we suggest that damage to nerve tissue occurs probably in two stages. First, the outer covering of neurons, namely, the myelin sheath, is damaged; this exposes the nerve tissue. In the second stage, neuronal damage occurs with relative accumulation of self-proteins, which cannot be readily hydrolyzed, such as prion proteins. Injection of brain tissue into experimental animals causes a neurological autoimmune disorder called EAE, and the histological appearance is characterized by vacuolation due to destruction of myelin, with subsequent degeneration of neuronal tissues. As the disease progresses, coalescing vacuoles form larger cavities and give rise to a spongelike or spongiform appearance. A highly encephalitogenic peptide with the following sequence, FSWGAEGQK, has been isolated from bovine myelin (*12*). We have used this sequence to search the Genbank and SwissProt databases for similar sequences

Table 1. Comparison of amino acids of bovine myelin and prion to microorganisms from Genbank and SwissProt, which have similar sequences in other proteins

Source	Amino Acids	Positions	Locations
Bovine myelin comparisons			
Bovine myelin	LSRFSWGAE	110–118	
Acinetobacter calcoaceticus	ISRFAWGEV	41–49	4-carboxy-muconolactone decarboxylase
Agrobacter tumefaciens	YTRFTWGAP	693–701	Beta-glucosidase
Ruminococcus albus	YTQFEISAE	274–282	Beta-glucosidase
Prion proteins comparisons			
Bovine prion	NMKHVAG	119–125	
Human prion	NMKHMAG	108–114	
Escherichia coli	QMKQMSG	340–346	*E. coli* signal recognition protein
Escherichia coli	NMKQMSG	118–124	*E. coli* colicin M

Abbreviations: A, alanine; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

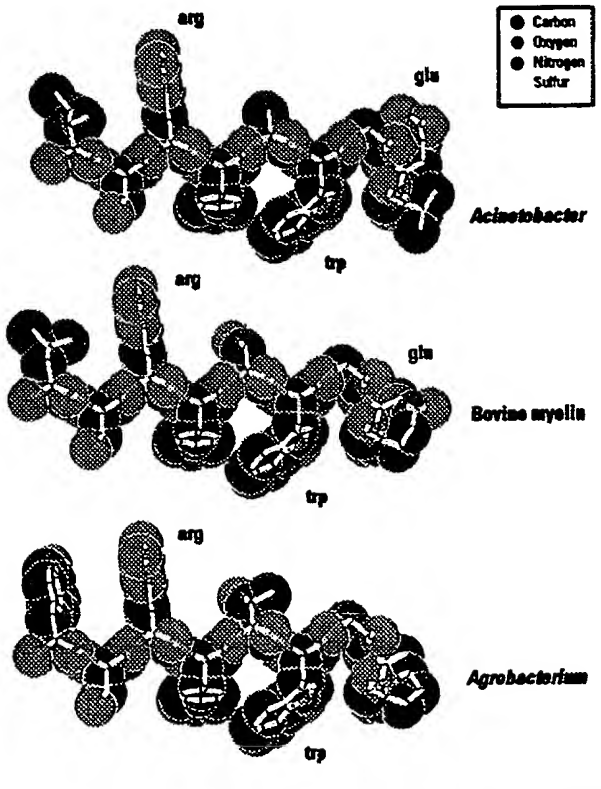

Figure 1. Comparison of space filling models, using Alchemy III (Tripos ASSOC Inc, St. Louis, MO) of *Acinetobacter calcoaceticus*, bovine myelin, and *A. tumefaciens*, which shows molecular mimicry between myelin and bacterial antigens. The immune response to these bacteria, over time, may cause spongiform changes characteristic of chronic experimental allergic encephalomyelitis and neurological symptoms of bovine spongiform encephalopathy. Abbreviations: arg, arginine; glu, glutamic acid; trp, tryptophan.

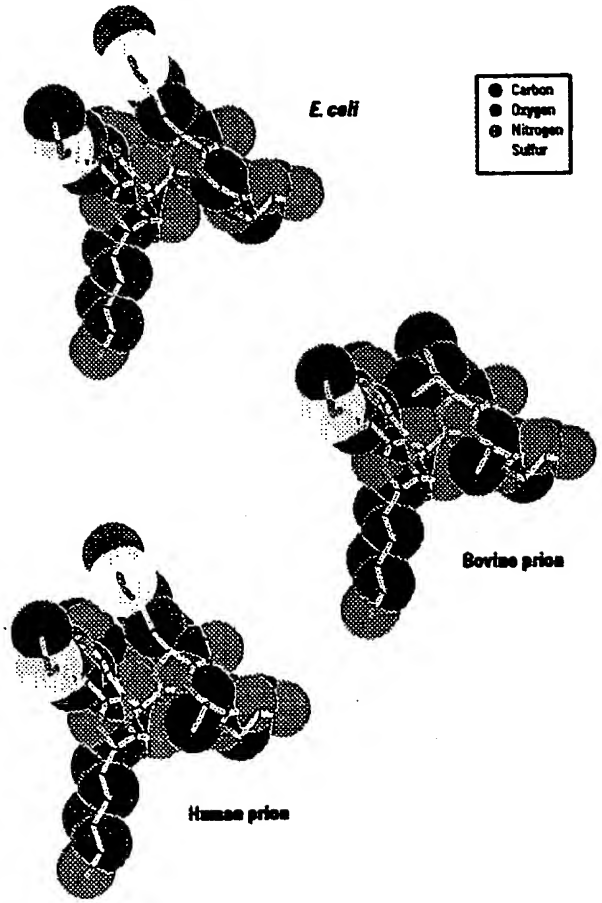

Figure 2. Comparison of space filling models of *Escherichia coli* signal recognition protein, bovine prion, and human prion. The nonaliphatic sequence of the prion molecule shows molecular mimicry with the *E. coli* recognition protein. The immune response to *E. coli* may contribute to the neuronal lesions found in spongiform diseases.

allowing for mismatches and found three microbes that show partial molecular mimicry to bovine myelin: *Acinetobacter calcoaceticus, Ruminococcus albus,* and *Agrobacterium tumefaciens* (Table 1).

Acinetobacter is a microbe found extensively in soil and water supplies, *Agrobacterium* is a plant pathogen causing galls, and *Ruminococcus* is found in the bowel flora of ruminants. The disease scrapie, which occurs in old goats and sheep, is also thought to belong to this group of diseases. Such animals are nibblers of shrubs, and it may not be that unexpected that they could consume galls containing large quantities of *Agrobacteria.*

The sequence in *Acinetobacter* contains a positively charged arginine (arg) and a negatively charged glutamic acid (glu), thereby forming an immunogenic epitope (Fig. 1). The host protein consisting of arginine-phenyl alanine-serine and tryptophan may bind immunocompetent cells and antibodies against the antigens of *Acinetobacter,* thereby causing damage to nervous tissue. Furthermore, the sequences in both *Acinetobacter* and *Agrobacterium* contain tryptophan (trp), an amino acid found to be necessary in producing EAE, because modification of the tryptophan residue led to loss of encephalitogenic activity (*12*). The biological activity of the encephalitogenic peptide remains after the protein is heated to 100°C for 1 hr or treated with 8 M urea (*12*), properties that it shares with the described properties of prion particles (*3*).

We have also used the bovine prion sequence NMKHVAG (*5,20*) to search the databases for similar sequences in microbes that may show partial molecular mimicry. Three sequences were found, all in the same microbe: NMKQMSG, *Escherichia coli* colicin M (Table 1); QMKNMGG, *E. coli* signal recognition protein (Fig. 2); and NMQHVAG, *E. coli* maltodextrin glucosidase.

If BSE is an autoimmune disease, elevated antibody levels to the bacteria showing molecular mimicry should be present during active phases, when acute phase reactants such as serum C-reactive protein levels are elevated (*21*). The pathological mechanism could be similar to mechanisms in rheumatic fever, rheumatoid arthritis (*22*), or ankylosing spondylitis (*23*) in which cross-reactive epitopes have been described in bacteria (site of infection), such as *Streptococcus pyogenes* (tonsillitis), *Proteus mirabilis* (cystitis), *and Klebsiella pneumoniae* (ileal Crohn's-like lesions) (*24*), respectively, which may act as autoimmune trigger factors in producing these diseases. Inadvertent feeding of cattle with supplementary foods containing meat and bonemeal that could have been exposed to these common bovine/ovine and environmental bacteria may have evoked immune responses leading to autoimmune disease.

The two theories have different economic implications: the prion-virus hypothesis proposes that cows/sheep (BSE/scrapie) are infected by the prion-virus agent; therefore, such animals should be culled with attendant financial costs. The autoimmune hypothesis proposes that neuronal damage is caused by immune processes similar to EAE following exposure in the gut to bowel bacteria carrying sequences resembling myelin and nervous tissues. Because the tissue damage is caused by self-proteins, namely autoantibodies, the affected animals are not infected, and treatment is to remove the offending cross-reactive antigenic bacteria from the bowel flora. Maternal transmission of BSE has occurred from dam to calf, but a similar situation is well described in human pathology in which pregnant women suffering from myasthenia gravis or thyrotoxicosis can transmit the disease via transplacental transfer of maternal IgG to their offspring. After birth, the neonates progressively recover from the disease as maternal IgG autoantibodies subside over time.

The autoimmune hypothesis predicts that BSE-affected animals should have elevated levels of antibodies to whole bacteria carrying cross-reacting self-antigens and to short peptides containing such sequences (bovine myelin, host encoded prion proteins); these could be helpful in establishing an early diagnosis.

Conclusions

The autoimmune hypothesis is a new theory that explains BSE by molecular mimicry between bacteria and brain tissue, but does not conflict with existing tenets of molecular biology. The theory should be tested by examining sera from BSE-affected cattle for antibodies to these bacteria.

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